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ELECTRON MICROSCOPIC OBSERVATIONS OF THE FINE STRUCTURE OF *TRICHOPHYTON RUBRUM DARK**

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ABSTRACT

With but one exception, the electron-dense lipid inclusions in mature hyphae, *T. rubrum dark* has the identical structure reported for other dermatophytes. The unique lipid accumulations increase steadily in amount and are responsible for the designation "dark." Initially, in young hyphae, the lipid material is seen in jagged, irregular tonoplasts. Gradually the lipid material becomes more concentrated, the membranes round up, and the lipid inclusions become more electron dense. The increase in size of the granules is the result of fusion of vesicles. In old hyphae the cytoplasm is filled with oval masses of lipid material.

Electron microscopy has shown that the ultrastructure of the dermatophytes is quite uniform. Species identification by electron microscopy is therefore not feasible; however, in this investigation electron microscopy has served as both a valuable taxonomic aid and a tool in determining the etiology of the purple coloration that appears in a growing colony of *Trichophyton rubrum dark*.

MATERIALS AND METHODS

T. rubrum dark was cultivated in Mycobiotic agar, pH 6.5 (Difco Laboratories, Detroit, Michigan). Generally, after 3 weeks of growth a purple coloration appears in the colony. Its intensity is such that at this time one can positively distinguish *T. rubrum dark* from *T. rubrum yellow*. The hyphae were fixed in osmium tetroxide [1], dehydrated in a graded series of alcohols, and embedded in Maraglas [2].

Sections, approximately 0.5 microns thick, were cut from Maraglas blocks and mounted on glass slides. The sections were stained with Paragon multiple stain (Paragon C. & C. Co., Bronx, N. Y.) and examined by light microscopy. This technique enabled us to determine the area in which the fungi were most concentrated. Fine sections were cut from those specific areas of the blocks and stained with uranyl acetate and lead citrate. Photographs were taken with an RCA EMU3F electron microscope.

OBSERVATIONS

An investigation of *T. rubrum* was previously undertaken but without any specific differentiation between "dark" and "yellow" varieties based on the presence of electron-dense lipid bodies [3]. Ordinarily the electron microscope is of no value as a taxonomic tool relative to dermatophytes because of the uniformity of the cytoarchitecture. *T. rubrum dark* definitely represents an exception. The fine-structure anatomy of young hyphae conforms with that of other species of dermatophytes which we have investigated [4–6]. The hyphae are elongate, filamentous, bi- and multinucleated structures. This typical morphology is illustrated in Figure 1. Mitochondria are conspicuous (Fig. 1*A*). The smooth endoplasmic reticulum, though generally sparse, is present in most hyphae (Fig. 2). Glycogen is an almost constantly occurring inclusion. It is scattered throughout the cytoplasm. The granules appear as electron-dense aggregations (Fig. 1*B*). The plasmalemma which bounds the cytoplasm is a delicate double-membrane sys-

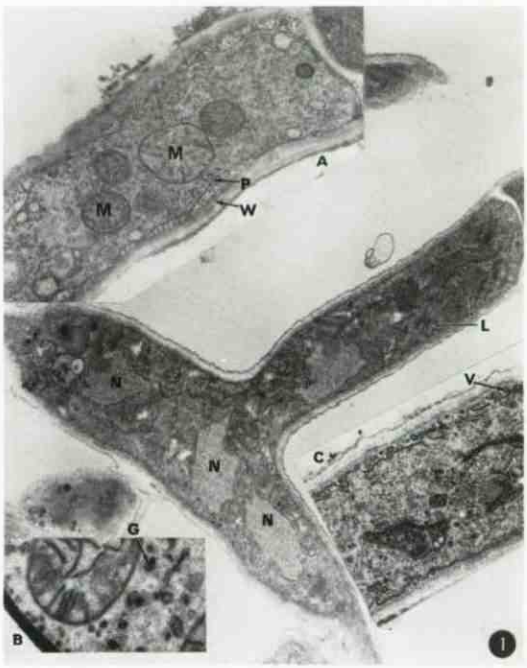

FIG. 1. Multinucleated (N) hyphae with irregular tonoplasts filled with lipid (L) (× 6,650). *A:* Cross-sectional profile of mitochondria at M. Double membrane plasmalemma at P. Broad cell wall at W (× 13,300). *B:* Glycogen aggregates at G (× 13,300). *C:* Distinct vesicles just outside of the plasmalemma shown at V (× 9,500).

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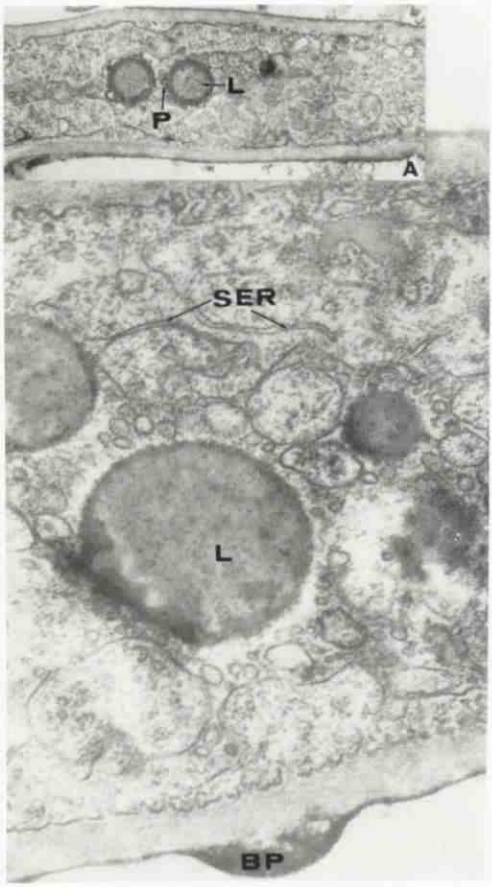

FIG. 2: Longitudinal profile of smooth-surfaced endoplasmic reticulum at SER. Lipid granule at L. A bulbous protrusion (BP) is present in the cell wall (× 33,500). *A:* Lipid granule (L) with small protrusions or vesicles at P (× 12,500).

tem (Fig. 1*A*). The cell wall (Fig. 1*A*) consists of a broad inner, slightly fibrillar, electron-lucid area and a thin outer, electron-dense delimiting area. Occasionally there are vesicular protuberances from the cell surface (Fig. 2). These peripheral sacs can be variably distended and show considerable heterogeneity. Of interest are the uniformly arranged, double-membrane bound vesicles situated just outside of the plasmalemma (Fig. 1*C*). These vesicles may be manifestations of ultrapinocytosis.

Although lipid is present in varying amounts in the other species of dermatophytes we have investigated [4–6], the cardinal exception to our initial generalization that the fine structure of *T. rubrum dark* is in conformity with all other dermatophytes is the presence of numerous, large, oval, electron-dense lipid bodies in the mature hyphae. These lipid inclusions represent the distinguishing feature which permits a positive identification. Lipid is visible in all figures, but in varying degrees. In young, one-week-old, metabolically active hyphae, homogeneous and only moderately opaque lipid material is concentrated in irregular, jagged tonoplasts (Fig. 1). By three weeks the hyphae mature and the lipid inclusions become especially numer-

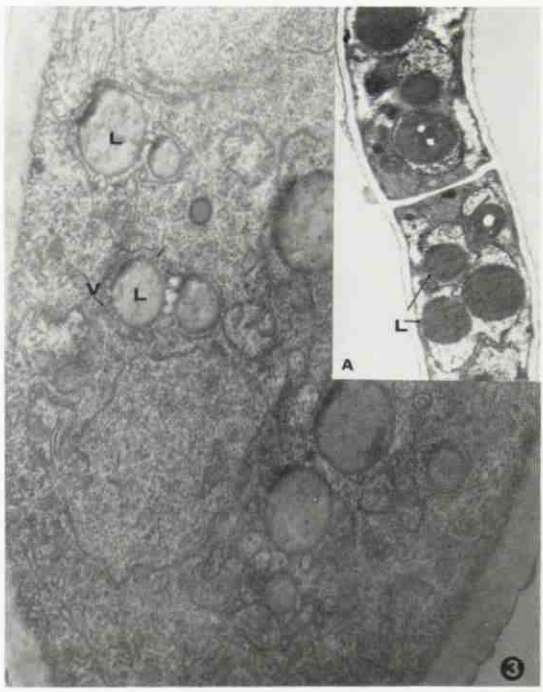

FIG. 3: A probable method of mature lipid granule formation (L)—vesicular coalescence—is indicated at arrows (× 25,000). *A:* Lipid granules (L) occupy the majority of the cytoplasm.

ous, spherical, and electron dense (Figs. 2,3). The small vesicles and jagged tonoplasts, characteristic of week-old hyphae, change radically in appearance in mature hyphae. In the latter there is a rounding up of the membranes and an increase in size. We theorize that the increase in size of lipid granules is the result of the numerous small contiguous vesicles (Figs. 2*A*, 3) becoming confluent with and pouring their contents into the large granule—a process perhaps homologous to the conversion of multilocular fat to the unilocular fat globule in man.

Thin-layer and gas-liquid chromatography studies are under way to determine the composition of these unique electron-dense bodies.

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